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INTERACTIONS OF ROD AND CONE SIGNALS IN THE MUDPUPPY RETINA

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SUMMARY

1. Interactions between rod and cone signals in mudpuppy retinal neurones were investigated by intracellular recording.

2. The mudpuppy retina contains one kind of rod (λ_{max} = 525 nm) and one kind of cone (λ_{max} = 572 nm). The responses of receptors can be distinguished on the basis of their spectral sensitivities.

3. Rod and cone responses have different time courses of recovery and absolute sensitivities. Differences between receptor responses can be used to describe inputs to interneurones.

4. There are two spectral classes of horizontal cells: L-type and C-type. L-type cells are hyperpolarized by rods and cones in varying proportion, with some cells receiving little rod input. C-type cells are hyperpolarized by rods and depolarized by cones.

5. Bipolar cell receptive field centres receive input from cones or from rods and cones. There is no correlation between the spectral properties of centre responses and their polarity.

6. Antagonistic surrounds of bipolar cells show cone or rod and cone sensitivity. They are believed to be generated by the L-type horizontal cells.

7. Some bipolar cells exhibit chromatic interactions between cone signals in the centre and rod signals in the surround, which resemble those observed between the signals of different spectral classes of cones in species known to possess colour discrimination.

8. Amacrine and on–off ganglion cells have L-type responses showing both rod and cone sensitivity.

9. It is proposed that interactions between rod and cone signals observed in mudpuppy also exist in primate retina and are at least partially responsible for certain psychophysical observations of rod–cone interactions.

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INTRODUCTION

It has long been known that the retinas of man and most other vertebrates contain two systems for visual perception: one (the scotopic) subserved by rods and the other (the photopic) by cones (Schultze, 1866). These two systems are usually thought of as completely independent of one another. It is well known that they function independently in most increment–threshold measurements, with the result that to a first approximation the threshold of the eye is determined simply by the system having the lower threshold (Stiles, 1944). A similar independence has been observed in the psychophysical phenomena known as the after-flash effect (Alpern, 1965) and lateral sensitization (Westheimer, 1970). However, the lack of interaction between the rod and cone systems under these conditions does not imply that they always function independently, since these experiments also fail to reveal interactions between the three kinds of cones in the human retina (Stiles, 1939; Alpern & Rushton, 1965; McKee & Westheimer, 1970) which nevertheless must exist in order to allow colour discrimination. The rod and cone systems have in fact been shown to interact under certain stimulus conditions (Hollins, 1971; Frumkes, Sekuler & Reiss, 1972; Makous & Boothe, 1974), and rods in dark-adapted observers can contribute to colour perception (Willmer, 1950; McCann & Benton, 1969).

Although there is much anatomical evidence for the convergence of rod and cone signals on to retinal interneurones (see, for example, Kolb, 1970; Lasansky, 1973; Kolb & Famiglietti, 1974; Scholes, 1975), it is not known to what extent they influence one another. The purpose of this study is to investigate interactions of rod and cone signals by intracellular recording. The mudpuppy was chosen for these experiments because it has large retinal cells that are easily penetrated by micropipettes (Werblin & Dowling, 1969), and because it contains rods and cones in approximately equal numbers (Palmer, 1912). Since it has only one rod and one cone pigment, whose absorption spectra are known (Liebman, 1972; P. K. Brown, unpublished), it is possible to distinguish the responses of the photoreceptors on the basis of their spectral sensitivities. The physiological differences between rod and cone responses can then be used to describe the interactions of their signals in retinal interneurones. The principal conclusion of this investigation is that rod and cone signals in mudpuppy interact to produce colour-opponent responses which are similar to those generated by the signals of different spectral classes of cones in vertebrates known to possess colour vision.

A preliminary report of some of these results has already appeared (Fain & Dowling, 1973).

METHODS

Preparation and dissection. Mudpuppies (*Necturus maculosus*) 20–25 cm long were kept in shallow, aerated tanks in a darkened 4–6° C cold room. Animals were further dark-adapted in a light-tight container the day before the experiment. They were decapitated and their eyes enucleated under dim red illumination, and the cornea and lens were removed under a dissecting microscope with the aid of light which passed through a Wratten No. 29 (red) filter. The eyecup was placed in a humid chamber in a light-tight Faraday cage and centred in the test beam of the photostimulator using dim red (680 nm) illumination. A steady stream of moist oxygen was gently blown over the retina.

Electrical recording. Extracellular (e.r.g.) recordings were made with cotton wick or silver–silver chloride electrodes placed in the vitreous. Moist cotton, on which the eyecup rested, served as the reference electrode. Intracellular recordings were made with micropipettes pulled from capillary tubing (type 7740, Corning) on a Livingston puller (F. S. Hockman, Media, Pa., U.S.A.) modified with a mechanical release. Pipettes were filled either with 1 M potassium acetate or 2 M potassium chloride by boiling at 80–90° C under a partial vacuum or by the glass-fibre method (Tasaki, Tsukahara, Ito, Wayner & Yu, 1968). Satisfactory electrodes had resistances between 200–800 MΩ measured in the vitreous.

Pipettes were driven into the retina at an angle of 45° with an hydraulic microdrive (David Kopf Instruments, Tujunga, Calif., U.S.A.). Signals were led into a high impedance, negative-capacitance pre-amplifier (ELSA-3, Electronics for Life Sciences), and high frequency noise was attenuated with a single passive low-pass filter whose time constant was either 3 or 7 msec. The responses of cells whose amplitudes were used to construct spectral sensitivity curves were negligibly attenuated by these filters. Responses were recorded either on 35 mm film or on a Brush recorder (Gould Inc., Cleveland, Ohio, U.S.A.).

Photostimulator. The eyecup was illuminated with one of two optical stimulators which have been previously described (Dowling & Ripps, 1971; Nelson, 1973). Both could be used to project test and adapting beams either of diffuse light or of spots and annuli whose radii, position, duration, intensity, and spectral composition could be independently controlled. The images of the stimuli from the two beams were monitored externally so that they could be positioned with respect to one another without light-adapting the retina. The last lens in the light path of both stimulators had a small diameter and long focal length, so that the light illuminating the retina was nearly collimated. The greatest divergence of the light rays from parallel was calculated to be less than 5° in the first stimulator, and less than 2° in the second.

Neutral density wedges (type M carbon coating, Eastman Kodak) and neutral glass absorption filters (Fish-Schurman Corp., New Rochelle, N.Y., U.S.A.) used in the two stimulators were calibrated *in situ* for variations in density across the spectrum using the monochromatic light provided by the stimulator, a photomultiplier tube placed in the position normally occupied by the eyecup, and a lock-in voltmeter (Brower Laboratories, Waltham, Mass., U.S.A.). The densities of some of the filters were also calibrated with a Cary recording spectrophotometer, and the two calibrations agreed within ± 0·05 log. units. Interference filters (Baird Atomic) used in both stimulators were calibrated on the recording spectrophotometer. The band width at half-peak transmittance for these filters was uniformly less than 10 nm. A monochromator was used in the test beam of the first stimulator, and its settings were adjusted with the calibrated interference filters.

The relative quantum flux incident on the eyecup at the wave-lengths used for spectral measurements was determined by placing a photodiode (Pin-5, United

Detector Technology Inc., Santa Monica, Calif., U.S.A.) in the position normally occupied by the eyecup. The diode was calibrated for relative quantum sensitivity by the manufacturer, and this calibration was verified by the author against a thermopile (Kipp & Zonen, Delft, Netherlands). Since this diode is quite sensitive to infra-red illumination, care was taken to ensure that leakage of I.R. from neutral or interference filters did not produce spurious readings. I.R. blocking filters (either KG-1 or KG-3, Schott-Jena) were placed in the beams during diode measurements, and a second set of filters was introduced to test for leakage. If, for a given measurement, the ratio of the diode current with the I.R. filters in the beam to that without was equal to the transmittance of the filters at the appropriate wave-length, then it was assumed that I.R. leakage was negligible.

The absolute intensity of the light source was calibrated with the Pin-5 diode as previously described (Fain & Dowling, 1973). With the beam unattenuated, the intensity at 550 nm in the test beam of the first stimulator was $1{\cdot}8\ \mu W/cm^2$ (or $5{\cdot}0 \times 10^{12}$ quanta.cm^{-2}.sec^{-1}) and that in the test beam of the second was $3{\cdot}3\ \mu W/cm^2$ (or $9{\cdot}1 \times 10^{12}$ quanta.cm^{-2}.sec^{-1}). The lamps in both stimulators were driven by regulated power supplies, and lamp intensity did not vary during an experiment or from one experiment to the next by more than $\pm 10\ \%$.

Procedure. An extracellular electrode was first lowered into the vitreous to measure the maximum amplitude of the e.r.g. The e.r.g. was monitored continually throughout the experiment; when its maximum amplitude diminished, the preparation was discarded. The extracellular electrode was then withdrawn, and a micropipette was lowered into the vitreous and positioned close to the centre of the eyecup under dim red illumination. The retina was further dark-adapted for 10–15 min before beginning the penetration, during which time the threshold of the b-wave stabilized. The micropipette was then advanced in steps of 2 or 3 μm, and the table on which the preparation rested was gently tapped to facilitate impalement. Flashes of diffuse white or 550 nm light were given at 7 sec intervals, whose intensities were adjusted so that the responses of cells could be elicited without light-adapting the retina (the 550 nm flash delivered 5×10^8 quanta.cm^{-2}). Penetration of a cell was signalled first by a sudden negative shift in potential and then by the appearance of a light-evoked response. Two lines of light $40 \times 800\ \mu$m were swept across the retina in directions perpendicular to one another to locate the centre of the cell's receptive field. Spots (either 160 or 170 μm radius) and annuli (either 160 μm inner $\times$ 240 μm outer radius or 250 μm inner $\times$ 500 μm outer radius) were used to characterize and identify the responses according to the physiological criteria given by Werblin & Dowling (1969). The dimensions of these stimuli were chosen to correspond with the dimensions of bipolar cell receptive fields (Werblin, 1970). Responses were occasionally recorded which could not be clearly identified according to these criteria. These are not included in the Results.

Spectral sensitivities were determined from the quantum flux necessary to produce a criterion response as estimated from the intensity–response curves at a number of different wave-lengths (Naka & Rushton, 1966). The inverse of this number, corrected for differences in the quantum flux of the test beam at different wave-lengths, was normalized and its log plotted against wave-length. For receptors, horizontal cells, and bipolars, the amplitudes of the responses were used to construct intensity–response curves; however, for amacrine cells and ganglion cells, the variation in amplitude spanned a narrow range of intensities (Werblin, 1971). The absolute latencies of these responses varied over a much wider range and so provided a more convenient measure of relative sensitivity (Werblin & Dowling, 1969). Relative sensitivity curves are thought to be accurate within about 0·1 log unit.

RESULTS

Spectral sensitivity curves were obtained from sixty-three cells. Nine of these were identified as photoreceptors, thirty as horizontal cells, thirteen as bipolar cells, six as amacrine cells, and five as ganglion cells.

Photoreceptors

The responses of photoreceptors could be identified by their relatively small receptive fields, short latencies, and fast rise times (Tomita, 1965; Werblin & Dowling, 1969). Fig. 1 compares the responses of two receptors and a representative horizontal cell to central and annular illumination. Both annulus and spot were presented at the same retinal illuminance, but the annulus was six times larger in area than the spot and so delivered six times as many quanta. In spite of this difference, the annulus was much less effective in stimulating the receptors, although it was much more effective for horizontal cells. The responses of the receptors were subsequently identified as those of a rod (upper traces) and a cone (middle traces) from their spectral sensitivities (see below). The response of the rod to the annulus may reflect interactions between rods which have been observed in other species (Schwartz, 1973; Fain, 1975). The responses in Fig. 1 should be compared to those in Fig. 3 of Werblin & Dowling (1969), who based their identification of receptor and horizontal cell responses on intracellular staining.

Spectrophotometric measurements have shown that the mudpuppy retina contains just two photopigments: a single porphyropsin with maximum absorbance at 525 nm, and a single A_2 cone pigment with maximum absorbance at 572 nm (Crescitelli, 1958; Brown, Gibbons & Wald, 1963; Liebman, 1972; P. K. Brown, unpublished). Since all mudpuppy rods appear to contain the 525 nm pigment and all cones the 572 nm (Liebman, 1972), spectral sensitivity curves could be used to identify the responses of photoreceptors.

Of the nine receptors for which spectral sensitivities were obtained, four were rods and five cones. The relative spectral sensitivities of two representative rods (*A*) and cones (*B*) are plotted in Fig. 2. Since the intensity–response curves for photoreceptors had the same shape at different wave-lengths, the selection of a criterion response did not affect the shapes of the spectral sensitivities. The continuous curves in Fig. 2 are the log relative absorbances of mudpuppy rod (*A*) and cone (*B*) pigments obtained from macrospectrophotometry on whole mudpuppy retinas by P. K. Brown (unpublished). Each curve is the mean log relative absorbance calculated from four determinations.

A description of the methods used in the absorbance measurements has

been given previously (Brown *et al.* 1963). In the mudpuppy retina both rod and cone pigments are relatively stable in hydroxylamine, so that the absorbances of both could be determined without appreciable contamination from bleaching intermediates (for $\lambda \geqslant$ about 460 nm). The curves in Fig. 2 are the difference spectra obtained by selectively bleaching first the

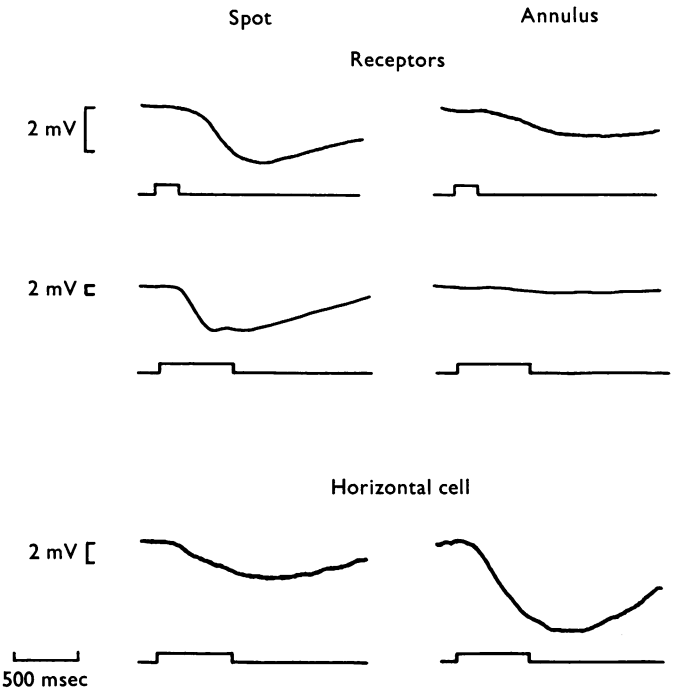

Fig. 1. Comparison of receptive fields of receptors and horizontal cells. Stimuli were a spot 160 μm in radius and an annulus 250 μm inner × 500 μm outer radius which were presented at the same retinal illuminance. Upper traces are from a rod, middle traces a cone, and lower traces from a C-type horizontal cell. Responses of L-type horizontal cells were similar. Records were retouched to improve contrast.

cone pigment with long wave-length light and then the remaining rod pigment with short wave-length radiation (cf. Brown & Wald, 1963).

The good agreement between the photoreceptor spectral sensitivities and the log relative absorbance curves in Fig. 2 suggests that the effective concentration of the photopigments in mudpuppy rods and cones is not as high as previously supposed. The microspectrophotometric measurements of Liebman (1972) imply a specific density at λ_{max} of 0·016/μm for both mudpuppy pigments, giving a peak absorption through the length of the

outer segment of 67 % for the rods and 59 % for the cones (Brown *et al.* 1963). However, spectral sensitivity data are consistent with photopigment absorption curves based on peak specific densities of no more than 0·01/μm (that is, on peak absorptions of no more than 50 % for rods and 43 % for cones). Absorption curves based on the specific density given by Liebman are much broader than the spectral sensitivity data. The reason for this

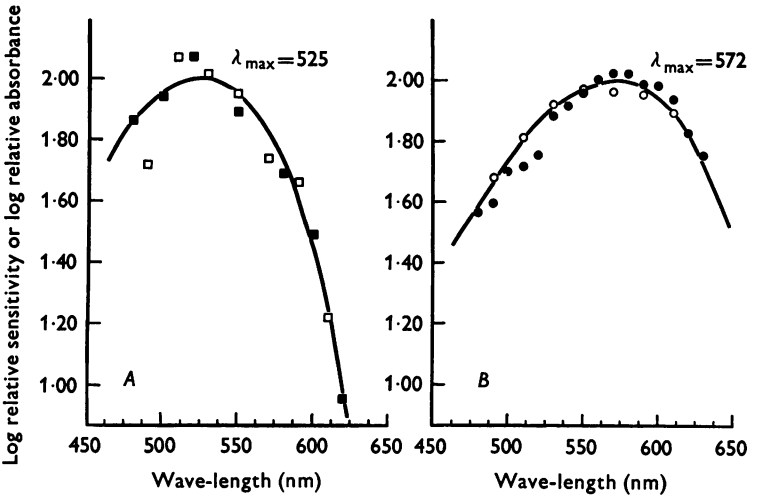

Fig. 2. Spectral sensitivities of photoreceptors, plotted against the relative absorbance of rod and cone photopigments obtained from macrospectrophotometry on whole mudpuppy retinas. *A*, log relative sensitivity of two cells classified as rods (squares) and rod pigment log relative absorbance (curve). *B*, log relative sensitivity of two cells classified as cones (circles) and cone pigment log relative absorbance (curve).

discrepancy is unknown. Photoreceptors were penetrated in the central region of the retina where, in other species, the long axis of their outer segments is perpendicular to the surface of the retina (Laties, Liebman & Campbell, 1968) and hence parallel to the incident light beam. However, it is possible that during dissection or penetration the outer segments were tilted away from their normal orientation.

The responses of a rod and a cone to 0·2 sec flashes of diffuse illumination are compared in Fig. 3. The rod (Fig. 3*A*) and the cone (Fig. 3*B*) both respond with graded hyperpolarizations. Although for a given response amplitude, the latency and rise time are much shorter in cones than in rods, for a given intensity the two have comparable wave forms at the onset of the response. Both show an initial transient to flashes of diffuse

light which is similar in wave form to that previously described for turtle cones (Baylor, Fuortes & O'Bryan, 1971) and for gecko rods (Kleinschmidt, 1974). These transients are somewhat smaller (and occasionally absent) in the mudpuppy receptors, perhaps because the retina becomes partially

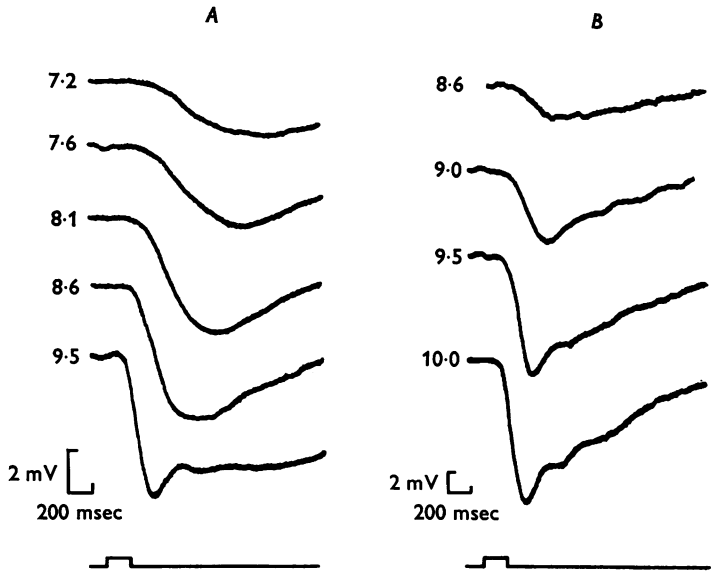

Fig. 3. Comparison of responses from a rod (*A*) and cone (*B*). Stimuli were 180 msec flashes of diffuse light at 510 nm for the rod and 600 nm for the cone. Relative absorbance curves of Fig. 2 were used to convert the intensities of the flashes into equivalent intensities at the λ_{max} of the rod or cone visual pigment. These are given to the left of each response in units of log incident quanta.cm^{-2}.flash^{-1}. Records were retouched to improve contrast.

anoxic during the course of the experiments (Baylor *et al.* 1971). The maximum amplitude of receptor responses (V_{max}) ranged from 3·5 to 21 mV for rods and 5 to 14·5 mV for cones.

Although the wave forms of mudpuppy rod and cone responses are similar in some respects, they differ strikingly in their time courses of recovery. The rod responses in Fig. 3*A*, for example, return to the base line much more slowly than those of the cone (Fig. 3*B*), whether the two are compared at the same amplitude of response or at the same stimulus intensity. Fig. 4 illustrates the slow time course of rod recovery for a different cell. Even at the dimmest intensity, the rod has not completely recovered after 10 sec. In addition to different recovery times, rods and cones have

different absolute sensitivities. The most sensitive dark-adapted mudpuppy rods are over 1·4 log units more sensitive to diffuse light than cones (Fain & Dowling, 1973). These differences, together with the spectral sensitivity curves, will be used to characterize the interactions of rod and cone signals in retinal interneurones.

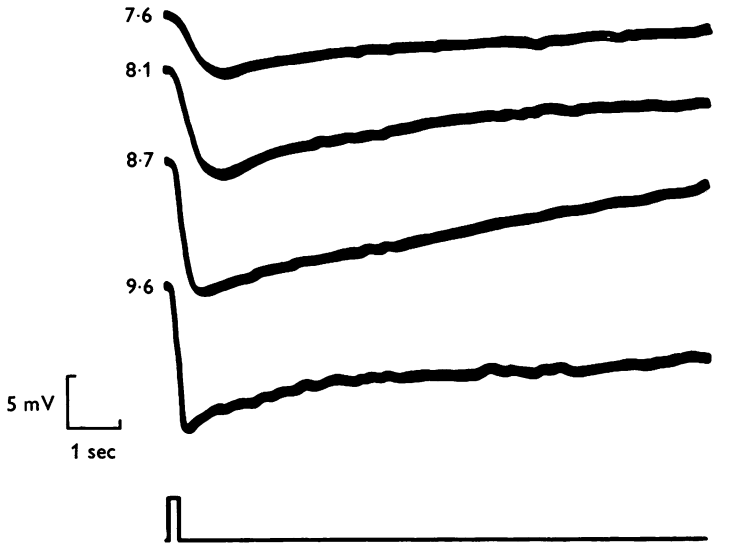

Fig. 4. Responses illustrating the slow time course of rod recovery. Stimuli were 190 msec flashes of diffuse light at 550 nm given at 25 sec intervals. As in Fig. 3, the intensities of the flashes were converted into equivalent intensities, and these are given to the left of each response in units of log incident quanta . cm^{-2} . $flash^{-1}$. Records were retouched to improve contrast.

There is apparently little interaction between rods and cones themselves, since for flashes of diffuse illumination the peak amplitudes of receptor responses obey the principle of univariance (see Fig. 2): response amplitudes depend only on the number of quanta absorbed and not on their wave-length (Naka & Rushton, 1966). This has been shown not to be true for turtle cones (Fuortes, Schwartz & Simon, 1973), which are apparently inhibited by L-type horizontal cells that receive input from more than one spectral class of photoreceptor (Fuortes & Simon, 1974). In the mudpuppy the L-type horizontal cells receive input from both rods and cones (see below), so that inhibition from these cells would also produce chromatic interactions in the photoreceptors. Small interactions of this kind were observed in cones, as the records of Fig. 5 demonstrate. The responses in this Figure are to flashes of the same equivalent intensity (that is, which bleached

the same number of cone pigment molecules) at 500 and 630 nm. The responses have different wave forms, that to the short wave-length having a more pronounced transient and a somewhat faster decay to the base line. However, these differences do not alter the spectral sensitivity of the peak amplitude of the response, suggesting that chromatic interactions between cones and horizontal cells may not be functionally significant in mudpuppy.

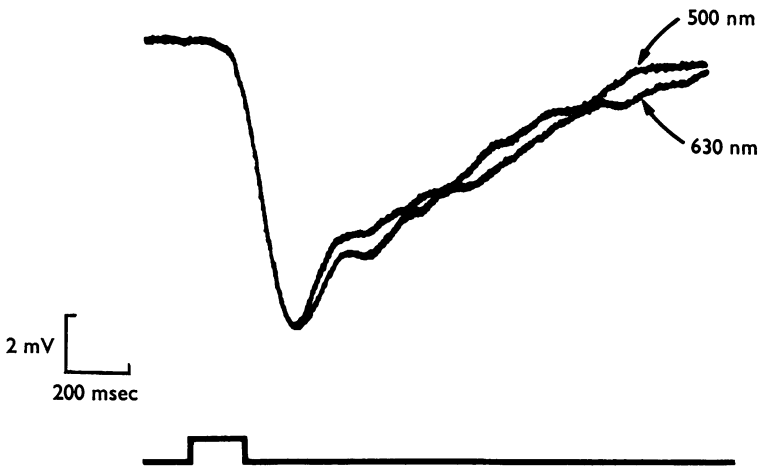

Fig. 5. Comparison of the wave form of cone responses to long and short wave-length illumination. Stimuli were 180 msec flashes of diffuse light which had the same equivalent intensity for the cone pigment (8×10^9 incident quanta.cm^{-2}.flash^{-1} at λ_{max}). Records were retouched to improve contrast.

L-type horizontal cells

The great majority of horizontal cells from which recordings were made were 'luminosity' or 'L-type' cells, receiving only hyperpolarizing input. Dark-adapted intensity–response curves of these cells resemble those recorded from cat L-type horizontal cells (Steinberg, 1969*a*; Niemeyer & Gouras, 1973), having different shapes at different wave-lengths. Curves for short wave-length stimulation consisted of two parts: a slowly rising segment at dim illuminations and a steeply rising part appearing at brighter intensities. Curves for long wave-lengths had only the steeply sloped component.

Since the intensity–response curves of mudpuppy L-type cells are not parallel along the abscissa, the shape of their spectral sensitivity curves will depend upon the voltage selected for the criterion response. Fig. 6 shows representative spectral sensitivities of a horizontal cell for three criterion values: 7·5, 10 and 15 mV. For the lowest (7·5 mV) criterion,

the spectral sensitivity follows the curve for the cone pigment log relative absorbance only in the red region of the spectrum. At about 525 nm, the sensitivity of the cell (dashed curve) begins to lie above the cone absorbance curve. Hence for low criterion values there are two components to

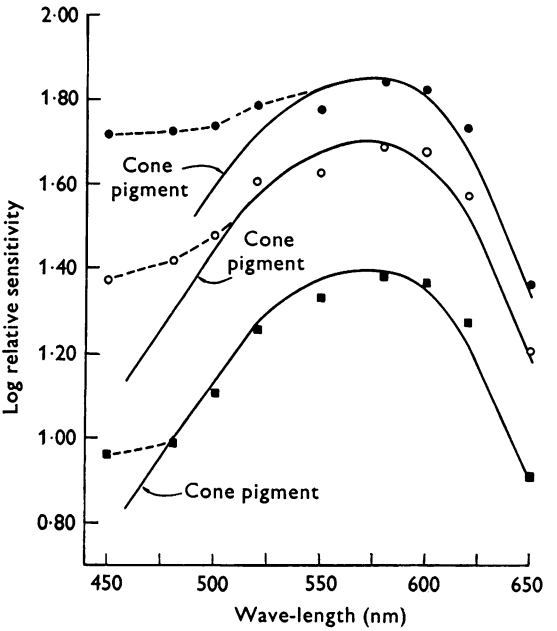

Fig. 6. Spectral sensitivity of a dark-adapted L-type horizontal cell as a function of criterion response. Data give the log relative sensitivities for criterions of 7·5 (●), 10 (○), and 15 mV (■). Points for each criterion have been fitted in the long wave-length region with the cone pigment log relative absorbance (continuous curve). At short wave-lengths, the sensitivity of the cell (dashed curves) lies above the cone relative absorbance, indicating that the cell also received input from a second component.

the spectral sensitivity corresponding to the two segments of the intensity–response curves: the cone component and a second component which becomes prominent only in blue region of the spectrum. For the intermediate criterion (10 mV), these two components are still present, although the blue sensitivity is now less prominent. At higher criterion values, the spectral sensitivity follows the cone pigment absorbance curve even for short wave-length stimulation. The sensitivity at the 15 mV criterion diverges from the cone curve only at 450 nm. This difference could be due to some small remaining blue-sensitive component; however, it is possible that the cone absorbance curve used in this Figure (which is taken from Fig. 2*B*)

does not reflect the true absorbance of the cone pigment at 450 nm. The absorption of light by the oxime following the bleaching of the cone pigment would give a difference curve that was narrower than the true pigment absorbance at short wave-lengths.

If the blue-sensitive component of the L-type response represented the input of a second receptor, then its contribution to the spectral sensitivity should be enhanced by chromatic adaptation with red light. An experiment of this kind is illustrated in Fig. 7. The data in the upper part of the Figure give the dark-adapted spectral sensitivity of an L-type cell. The lower curve gives the sensitivity of the same cell for the same criterion response measured in the presence of a 706 nm background illumination. The background depressed the peak sensitivity of the cell by about 2·1 log units, but it enhanced the relative sensitivity in the short wave-length portion of the spectrum.

The blue-sensitive component is probably coming from the rods since, in the dark-adapted retina, it is associated with the slowly rising portion of the intensity–response curve, which is prominent only at short wave-lengths and for dim light intensities. Additional evidence implicating the rods comes from the wave form of the horizontal cell response. Since rod and cone photoreceptors show very different time courses of decay, horizontal cells receiving input from both kinds of photoreceptors should show two phases of decay. These are most readily observed for flashes of short wave-lengths. Fig. 8*A*, for example, shows the responses of an L-type horizontal cell to 0·2 sec flashes of 450 nm light at intensities increasing in steps of 0·5 log units from 8·3 to 10·3 log quanta.cm^{-2}. $flash^{-1}$. At the dimmest intensity (which would have produced a response of about 0·6 V_{max} in rods but only about 0·06 V_{max} in cones), the wave form of the horizontal cell response resembles that of a rod receptor at this illumination, showing only a slow, prolonged decay (cf. the rod response at 8·1 log quanta.cm^{-2}.$flash^{-1}$ in Fig. 4). At brighter illuminations, there is also a fast decay resembling that of the cones.

The slow decay is much more prominent for short wave-length illumination than for long wave-lengths. Fig. 8*B* illustrates the responses of another L-type horizontal cell to stimuli at 450 and 650 nm. The responses to 450 nm light are like those in Fig. 8*A*, whereas those to the 650 nm stimuli show only a fast decay. An attempt was made to measure the spectral sensitivity of the slow decay from these and other, similar records. Although it was clearly most sensitive in the short wave-length region of the spectrum, it was too small in amplitude to be used to make an accurate estimate of the wave-length of peak sensitivity.

The proportions of rod and cone input in an L-type horizontal cell can be estimated from the size of the slowly rising component in the intensity–

response curves and from the relative amplitudes of fast and slow decay. In all of the L-type horizontal cells whose spectral sensitivities were measured, the rod component was smaller than the cone, but the proportion of rod input varied from cell to cell. At first it was thought that this variation was due to differences in the sensitivity of the rods from

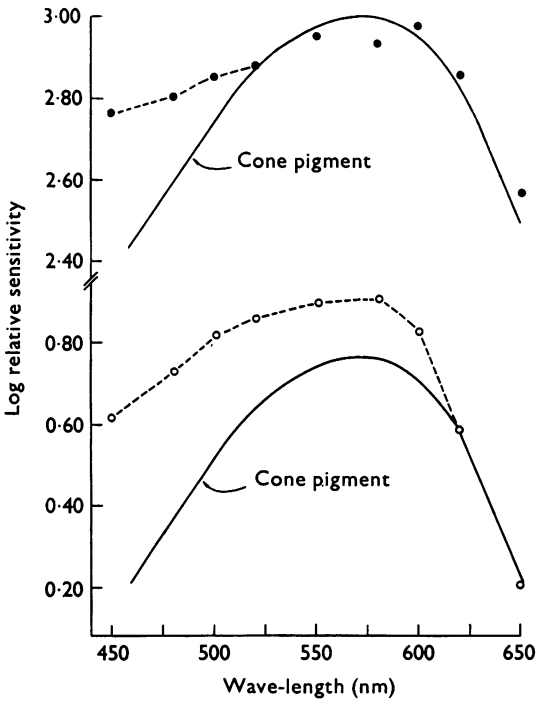

Fig. 7. Spectral sensitivity of L-type horizontal cell in the dark (●) and in the presence of a red background (○). Both curves were measured with diffuse light and are for the same criterion response. The peak sensitivity of the cell in the dark was arbitrarily assigned a log relative sensitivity of 3·0. The background was a 706 nm diffuse field of intensity $1{\cdot}5 \times 10^{12}$ incident quanta.cm^{-2}.sec^{-1}. It depressed the peak sensitivity of the cell by about 2·1 log units. Both sets of data were fitted with the cone pigment log relative absorbance (continuous curves) in the long wave-length region. At short wave-lengths; the sensitivity of the cell (dashed curves) is greater than that indicated by the cone pigment curve, and this discrepancy is enhanced by the red background.

preparation to preparation; however, no correlation could be found between the rod sensitivity in the e.r.g. of a given retina and the size of the blue-sensitive component measured in its horizontal cells. The variation in the proportion of rod input may have been due to differences in the state of adaptation of the retina from preparation to preparation, but this

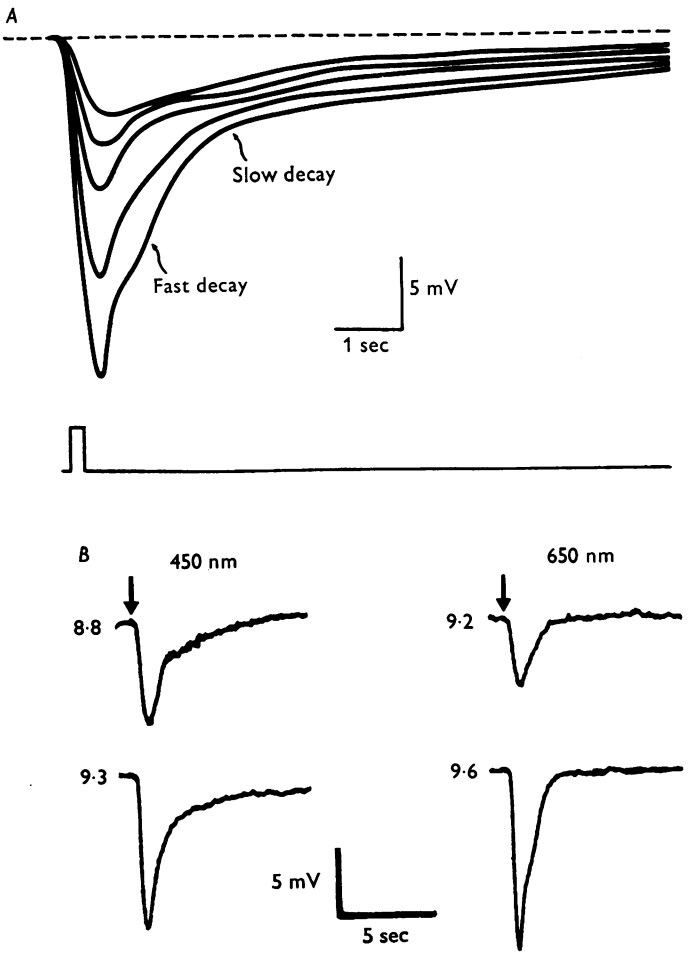

Fig. 8. Responses of dark-adapted L-type horizontal cells illustrating two phases of recovery. *A*, tracings of responses of an L-type horizontal cell to 190 msec flashes of diffuse light at 450 nm. Flashes were presented at intervals of 15 sec at intensities from 8·3 (uppermost trace) to 10·3 (lowermost trace) log incident quanta . cm^{-2} . $flash^{-1}$ in steps of 0·5 log unit. Dashed line indicates the resting potential of the cell in the dark. *B*, responses of a second L-type horizontal cell to 190 msec flashes of diffuse light at 450 and 650 nm. Numbers to the left of responses give the intensity in log quanta . cm^{-2} . $flash^{-1}$. Arrows indicate beginning of flashes. Responses at 450 nm have both fast and slow decays, whereas those at 650 nm show only the fast decay.

does not seem likely since in every case the retinas were maintained as fully dark-adapted as possible.

Among the twenty-nine L-type cells, there were five which gave little evidence of any rod input in the dark-adapted retina. These cells showed

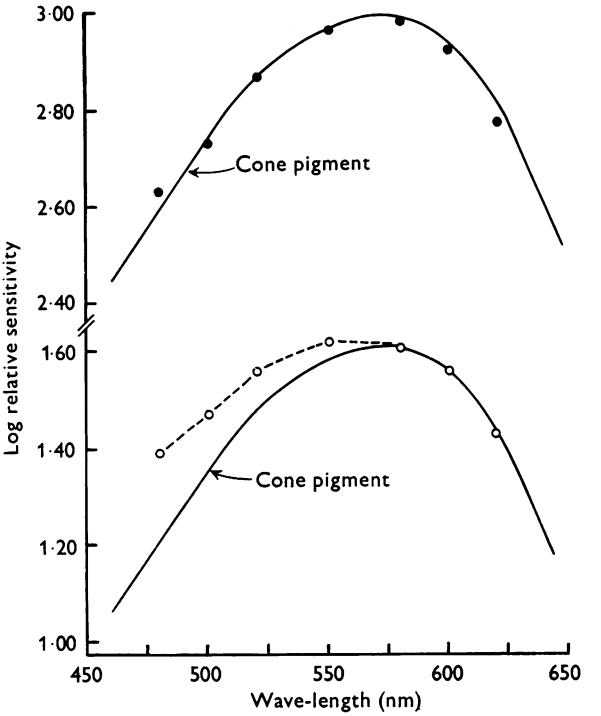

Fig. 9. Spectral sensitivity of L-type horizontal cell showing little rod input. Figure shows for the same criterion the spectral sensitivity of the cell in the dark-adapted retina (●) and in the presence of a diffuse, 706 nm background light (○) whose intensity was $1{\cdot}5 \times 10^{12}$ incident quanta.cm^{-2}.sec^{-1}. As in Fig. 7, the peak log sensitivity of the cell in the dark was arbitrarily assigned a value of 3·0. The sensitivity of the cell can be satisfactorily described by the cone pigment log relative absorbance (continuous curve) in the dark, but in the presence of the background it is more sensitive at short wavelengths than indicated by the cone curve.

little difference in the slopes of their intensity–response curves in the blue and red, and no blue-sensitive component in their spectral sensitivity curves, even for low criterion responses. They also showed little rod decay. The dark-adapted spectral sensitivity of such a cell is shown as the filled circles of Fig. 9. The relative sensitivity of this cell was close to the cone relative absorbance curve even at 480 nm (the sensitivity at 450 nm was

not measured). However, the relative sensitivity was enhanced in the blue by adaptation with a 706 nm background illumination (open circles). Thus, even this cell appeared to have some rod input, although it was so much smaller than the cone that it was not readily observed in the dark-adapted retina.

A C-type horizontal cell

One horizontal cell behaved quite differently from the L-type cells described above. This cell had the receptive field of a horizontal cell (its responses to central and annular illumination are illustrated in the bottom traces of Fig. 1) and, in the dark-adapted retina, gave hyperpolarizing responses to flashes from every region of the spectrum. However, even the dark-adapted responses suggested the presence of a second, inhibitory component. In the presence of blue background illumination, this second component became a prominent part of the response.

This cell will be called a 'chromaticity' or 'C-type' horizontal cell, since its spectral properties resemble those of C-type cells in fish retina (Svaetichin, 1956). Its dark-adapted responses to flashes of approximately equal quantum fluxes at 490 and 590 nm are illustrated in Fig. 10*A*. The response at 490 nm is indistinguishable from those typically recorded from L-type horizontal cells. The 590 nm response on the other hand was biphasic, consisting of a transient peak followed by a relatively fast decay to the base line. Comparison of the two responses suggests that the longer wave-length stimulus generated a delayed depolarization which inhibited the hyperpolarizing component.

To investigate the nature of this inhibition, response amplitudes at various times after the beginning of the light flash (arrows of Fig. 10*A*) were used to construct spectral sensitivity curves. The relative spectral sensitivities for 225, 450 and 675 msec, all for the same criterion response and normalized to the same sensitivity at 490 nm, are given in Fig. 11*A*. These are compared to the rod pigment relative absorbance curve, which appears broader in this Figure than in Fig. 2*A* because sensitivity has been plotted on a smaller scale on the ordinate.

The spectral sensitivities of the responses are all narrower than the rod pigment relative absorbance curve. The hyperpolarization in the dark-adapted retina appears to be produced by input from rods, but it is inhibited by some process whose peak sensitivity is at longer wave-lengths than the λ_{max} of the rod pigment. The increase in the strength of the inhibition between 225 and 675 msec, which is evident from the shapes of the spectral curves in Fig. 11*A*, could also be inferred from the records of Fig. 10*A*, since the 490 nm response increased in amplitude between 225 and 675 msec but the 590 nm response became smaller. However, it is

apparent that this inhibition was present even at the earliest time at which responses were measured. It could not be determined whether it was present even earlier, since the amplitudes of the responses before 225 msec were too small to be used to measure spectral sensitivity. The

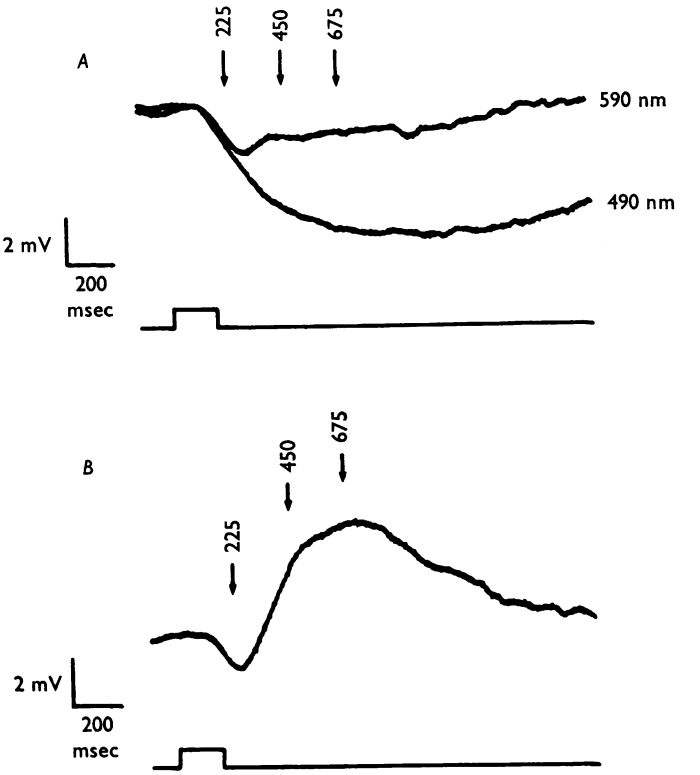

Fig. 10. Responses of a C-type horizontal cell. *A*, dark-adapted responses to 180 msec flashes of equal illuminance at 490 and 590 nm. Arrows indicate times after the beginning of the flash (in msec) at which response amplitudes were measured for the spectral sensitivities of Fig. 11*A*. *B*, responses to a 180 msec flash of diffuse 650 nm light in the presence of a 453 nm background.

latency of the response, which could also conceivably have been used to measure sensitivity, did not show a large enough variation for the stimulus intensities used in the experiment.

The relative sensitivity of the response at 590 nm, measured 675 msec after the beginning of the stimulus, was almost $2\frac{1}{2}$ log units less sensitive than predicted by the rod pigment absorbance curve. At even longer wave-lengths, the responses were so small that it was difficult to measure

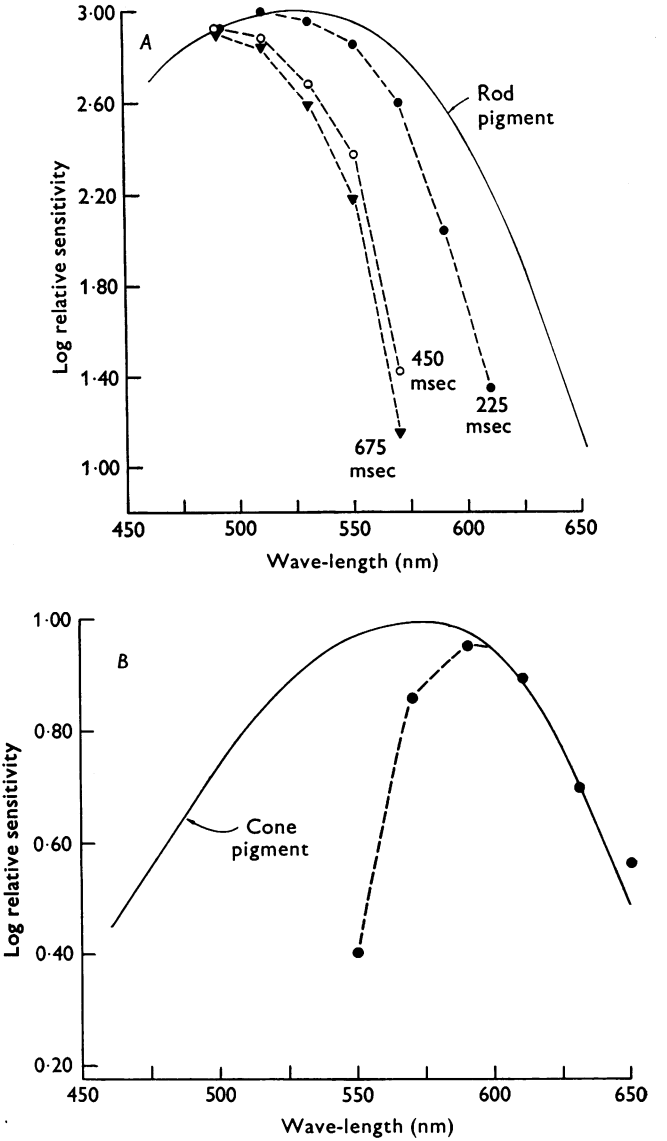

Fig. 11. Spectral sensitivities of C-type horizontal cell. *A*, dark-adapted spectral sensitivities for 1·4 mV criterion measured 225, 450 and 675 msec after the beginning of the flash. Sensitivities have been normalized at 490 nm and are compared to the rod pigment log relative absorbance (continuous curve). *B*, spectral sensitivity of the depolarizing component recorded in the presence of 453 nm background illumination. Responses were measured from the bottom of the hyperpolarizing notch to the peak of the depolarization and are fitted with the cone pigment log relative absorbance (continuous curve) in the long wave-length region.

their sensitivities. However they never became depolarizing in the dark-adapted retina.

Depolarizing wave forms could be observed in the presence of blue background illumination. The response of the cell to a 650 nm flash superimposed upon a 453 nm background is illustrated in Fig. 10*B*. At the onset of illumination there is a small, hyperpolarizing notch which had a relative spectral sensitivity (measured between 550 and 650 nm) which could be closely fitted with the rod pigment log relative absorbance curve. Hence it was probably generated by the same process that produced the hyperpolarizing component in the dark-adapted retina. This notch was followed by a depolarization, whose amplitude increased between 225–675 msec. The time course of the depolarization was thus consistent with the time course of the inhibition observed in the dark-adapted retina. However, it should be noted that the retina was undoubtedly light-adapted by the blue background, so that the time courses of the responses in Fig. 10 may not be entirely comparable.

Fig. 11*B* shows the spectral sensitivity for the peak depolarization of the response recorded in the presence of 453 nm background illumination. These data could be closely fitted with the cone pigment relative absorbance curve for wave-lengths greater than 600 nm. At shorter wave-lengths, the sensitivity was narrower than the cone pigment relative absorbance, probably because the hyperpolarizing component still present at this background intensity suppressed the depolarization. Stronger backgrounds should have reduced this remaining hyperpolarization, but the cell was lost before this could be investigated.

Bipolar cells

Bipolar cells were identified according to the physiological criteria given by Werblin & Dowling (1969) and Miller & Dowling (1970). The mudpuppy retina contains two kinds of bipolars, called 'hyperpolarizing' or 'depolarizing' according to the polarity of their response to central illumination (Werblin, 1970). In both, the responses of the centre are strongly inhibited by illumination of an annular surround. Bipolar cells could be separated into two further classes on the basis of their spectral sensitivities. Those showing predominantly cone sensitivity in the centre will be referred to as 'cone' bipolars, while those showing evidence of input in the centre from both kinds of receptors will be called 'rod-cone' bipolars.

Cone bipolar cells. Fig. 12 gives the relative spectral sensitivities for both the centre response and the surround inhibition for a hyperpolarizing bipolar. The sensitivity of the surround was measured with an annulus flashed in the test beam around maintained central illumination provided

by a white light from the adapting beam. Both centre and surround were maximally sensitive at about 575 nm and have been fitted with the relative absorbance curve for the cone pigment. In contrast to this cell, the spectral sensitivity of the depolarizing bipolar of Fig. 13 is not the same in both parts of its receptive field. As for the previous cell, the centre has been fitted with the absorbance curve of the cone pigment. However, the surround response has its peak sensitivity at shorter wave-lengths,

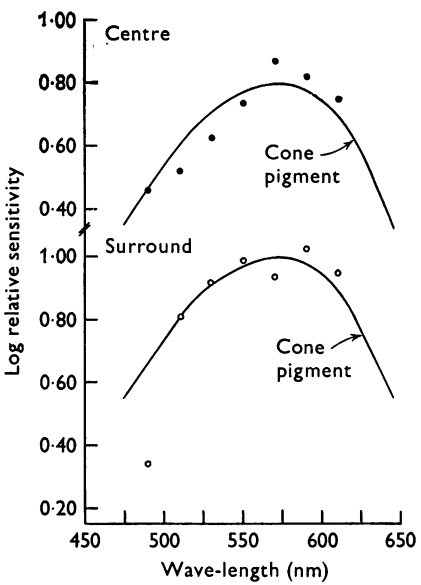

Fig. 12

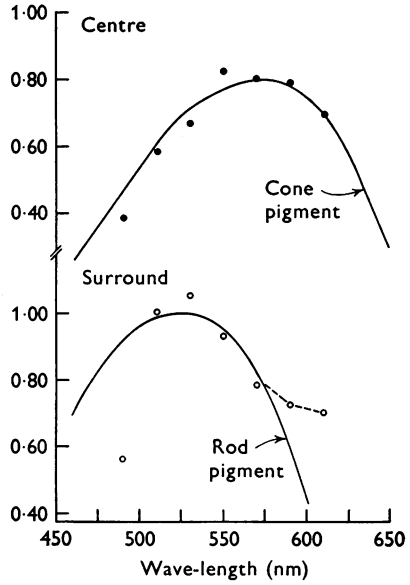

Fig. 13

Fig. 12. Spectral sensitivities of a hyperpolarizing bipolar cell. Centre (●) and surround (○) responses have both been fitted with the cone pigment log relative absorbance (continuous curves).

Fig. 13. Spectral sensitivities of a depolarizing bipolar cell. The centre response (●) has been fitted with the cone pigment log relative absorbance (upper curve) and the surround response (○) with the rod pigment log relative absorbance (lower continuous curve).

between 510 and 530 nm. It has been fitted with the rod pigment log relative absorbance curve in the short wave-length region of the spectrum. At 590 and 610 nm, its spectral sensitivity (dashed curve) lies somewhat above the rod pigment relative absorbance, suggesting that a second process may also contribute to surround inhibition in this cell. Spectral sensitivities of surrounds were measured in four bipolars: two showed predominantly cone and two predominantly rod sensitivity, with both 'rod'

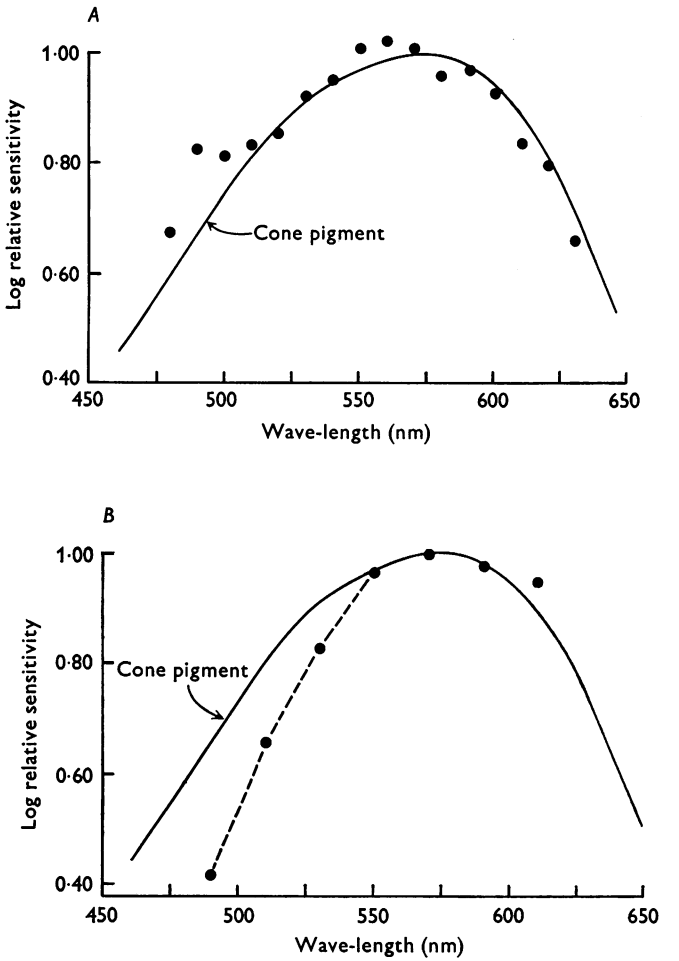

Fig. 14. Spectral sensitivities of bipolar cells to light which stimulated both centre and surround simultaneously. *A*, log relative spectral sensitivity of a depolarizing bipolar to full-field illumination, fitted with the cone pigment log relative absorbance (continuous curve). *B*, log relative spectral sensitivity of a hyperpolarizing bipolar measured with a 170 μm radius spot. The response was dominated by the centre but also showed evidence of input from the surround (see text). The data have been fitted with the cone pigment log relative absorbance (continuous curve) in the long wave-length region but are less sensitive than indicated by this curve at short wave-lengths. This difference was also observed in two other cells whose sensitivities were measured with diffuse light.

surrounds being somewhat more sensitive in the red region of the spectrum than indicated by the rod pigment relative absorbance.

In some bipolar cells, the centre response could not be completely isolated from surround inhibition, either because the spot was not properly centred, because it was larger in diameter than the centres of some bipolar cell receptive fields, or because the centre and surround of some bipolar cells overlap. The responses of these cells were dominated by the centre but always showed evidence of some inhibition from the surround, indicated by rapid time course of decay and the presence of an after-potential (Werblin & Dowling, 1969). The spectral sensitivities of the peak amplitudes of these responses were measured either with spot or full-field illumination. These fell into two groups. Fig. 14*A* is representative of two cells whose sensitivity could be satisfactorily fitted with the cone pigment relative absorbance curve. These cells resemble the cell of Fig. 12, in that they show no evidence for interaction between rod and cone signals even when (as in Fig. 14*A*) they are stimulated with diffuse light. In contrast, the cell of Fig. 14*B* is representative of three cells whose spectral sensitivities were narrower than the cone pigment relative absorbance.

The narrowing of the curve in Fig. 14*B* could be the result of a difference in the spectral composition of the centre and surround. If for example the centre received predominantly cone input but the surround were more sensitive than the centre at short wave-lengths (as for the cell of Fig. 13), then the sensitivity of the response when both centre and surround were stimulated together would be dominated by the cone pigment but would be narrower in the blue than the cone relative absorbance curve.

The interaction of rod and cone signals in the centre and surround of this bipolar is further suggested by the wave form of its responses to short and long wave-length illumination. The responses illustrated in Fig. 15 to 510 and 610 nm illumination have equal peak amplitudes but are otherwise quite different. The two at 510 nm have faster rise times than those at 610 nm, as if the cell were responding to a brighter intensity at this wave-length; but they then decay more rapidly to the base line. This faster decay is probably caused by the arrival of the surround inhibition, which is relatively more sensitive than the centre to short wave-lengths.

Since the centre response has a shorter latency than the surround (Werblin & Dowling, 1969), it should be possible to separate the effects of the two by measuring the responses of the bipolar cell at a number of times after the beginning of the light flash. Measurements at the times indicated by the arrows in Fig. 15 were used to construct intensity–response curves, from which spectral sensitivities were determined. These

are given in Fig. 16, all for the same criterion response and normalized to the same sensitivity at 610 nm.

The filled circles in this Figure give the sensitivity of the response at the earliest time for which it could be measured. Because of the longer latency of the surround, this first curve most nearly approximates the sensitivity of the centre free of surround inhibition. Since it can be closely fitted with

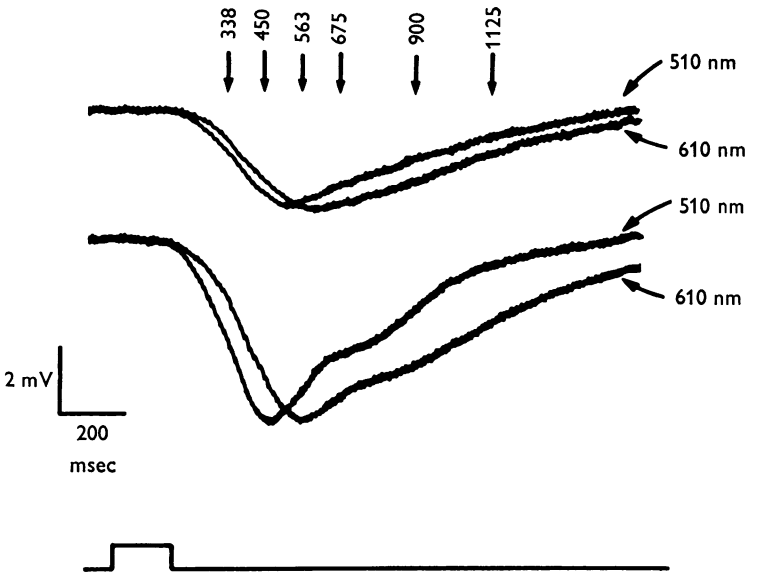

Fig. 15. Equal amplitude responses of the hyperpolarizing bipolar of Fig. 14*B* to short and long wave-length illumination. Stimuli consisted of 180 msec flashes at the following illuminances, in units of log quanta.cm^{-2}. flash^{-1}: 9·1 at 510 nm and 8·6 at 610 nm (upper traces) and 9·6 at 510 nm and 9·1 at 610 nm (lower traces). Arrows indicate times after the beginning of the flash (in msec) at which response amplitudes were measured for the spectral sensitivities of Fig. 16. Traces retouched to improve contrast.

the cone pigment relative absorbance curve, the centre response of this cell is apparently generated exclusively by cone input. At later times, the sensitivity becomes more and more narrow in the blue (dashed curves), indicating that the longer latency surround response is relatively more sensitive than the centre at shorter wave-lengths. Notice that the spectral sensitivity begins to narrow even before the bipolar cell response reaches peak amplitude. Since the surrounds of some cone bipolar cells receive rod input (Fig. 13), this narrowing is probably mediated by the rods.

Rod–cone bipolar cells. A second class of bipolar cells gave spectral sensitivity curves for central or full-field illumination which were quite different from those shown above. These cells were less frequently

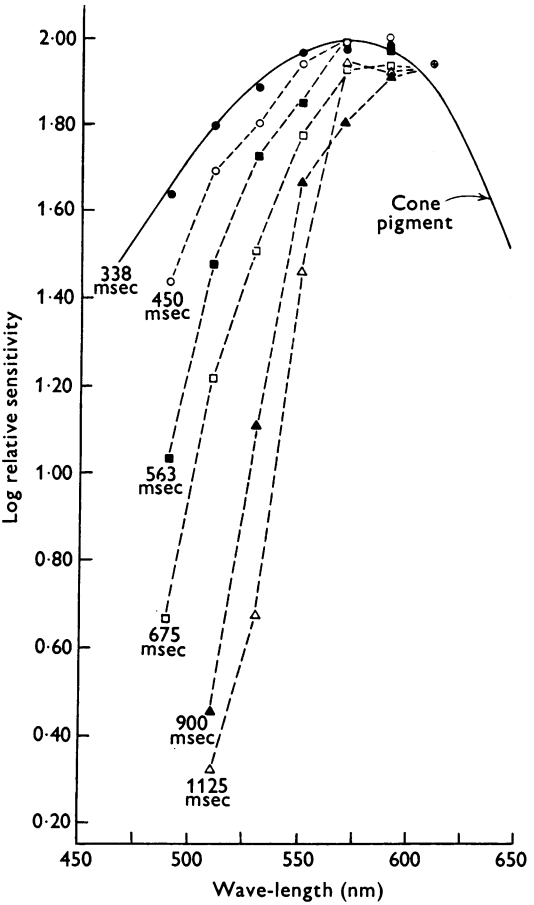

Fig. 16. Spectral sensitivity for the response of the hyperpolarizing bipolar of Fig. 15 as a function of time after the stimulus. Log relative spectral sensitivities for the same (2 mV) criterion were obtained from the amplitude of the response at the following times in msec after the beginning of the stimulus: 338 (●), 450 (○), 563 (■), 675 (□), 900 (▲), 1125 (△). Data for the various times have been normalized at 610 nm (⊕). The 338 msec data is fitted with the cone pigment log relative absorbance (continuous curve).

encountered than cone bipolars (spectral sensitivity curves were obtained from only two such cells), and no measurements were made on their surrounds.

The spectral sensitivity of the centre response of a hyperpolarizing

bipolar from this second class is shown in Fig. 17. The open circles give the measurements obtained upon first entering the cell. After these were completed, the cell abruptly increased both in absolute sensitivity and in peak amplitude, perhaps as the result of a sudden movement of the pipette. It was allowed to stabilize in the dark for 1–2 min, and then a

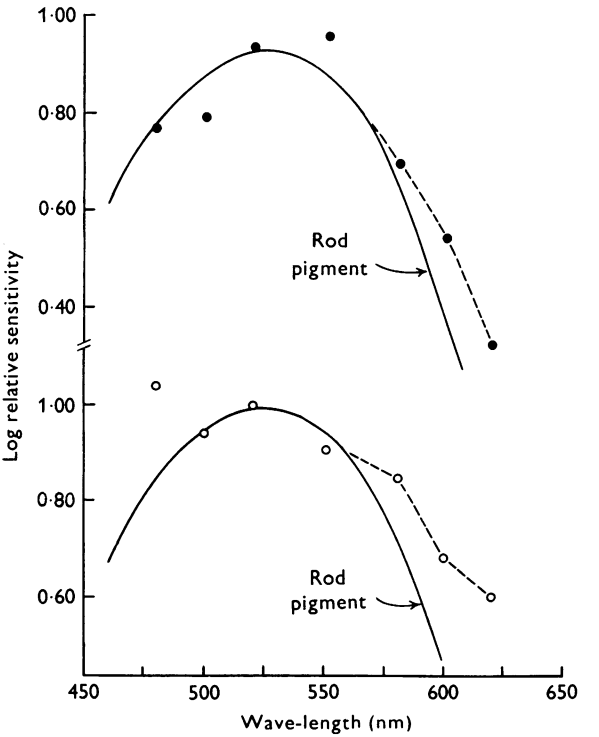

Fig. 17. Spectral sensitivity of the centre response of a dark-adapted hyperpolarizing bipolar cell. Data give the log relative sensitivities upon first entering the cell (○) and after an abrupt increase in sensitivity (●), both for the same criterion response. Both sets of data have been fitted at short wave-lengths with the rod pigment log relative absorbance (continuous curves).

second series of measurements was made (filled circles). The data for both spectral sensitivities have been fitted with the rod pigment log relative absorbance curve in the short wave-length region of the spectrum. At longer wave-lengths, the sensitivity of the cell (dashed line) lies above the rod pigment relative absorbance curve, suggesting that a second component with its spectral maximum at longer wave-lengths contributed to the response. A similar curve was obtained from a depolarizing bipolar using full-field illumination.

The long-wave-length component in these cells probably comes from the cones, since the responses of these cells exhibit a wave-length-dependent shift in wave form similar to that of the L-type horizontal cells. Fig. 18 gives representative centre responses for the hyperpolarizing bipolar of Fig. 17 at 480 and 620 nm. The responses at 480 nm show both

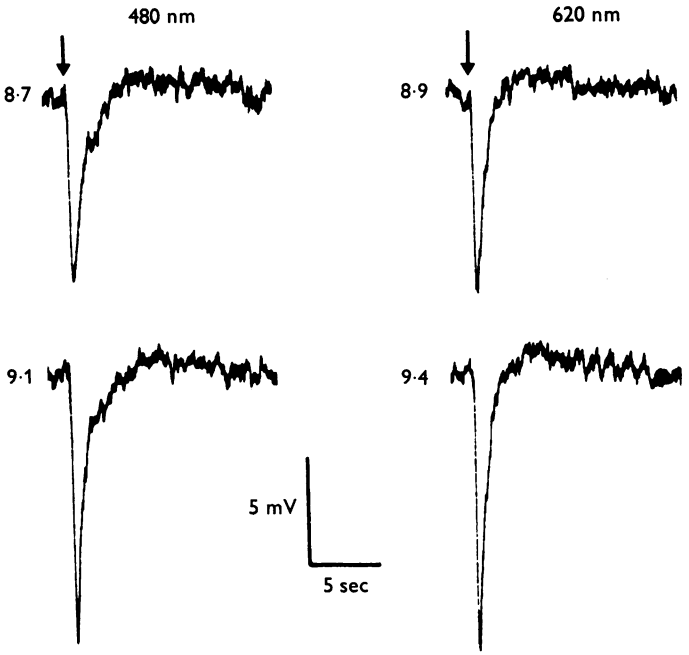

Fig. 18. Centre responses of the hyperpolarizing bipolar of Fig. 17 to light of long and short wave-lengths. Stimuli were 190 msec flashes of a 160 μm radius spot at 480 nm and 620 nm. Intensities of the flashes are given to the left of each response in units of log quanta.cm^{-2}.flash^{-1}. Arrows mark the beginning of the stimuli.

a slow decay characteristic of the rod receptors and a fast decay characteristic of the cones, whereas those at 620 nm show little or no slow decay. The small depolarizing overshoot at the cessation of illumination is probably due to a partial stimulation of the surround (Werblin & Dowling, 1969), which could also have contributed to the differences in the wave forms observed in these recordings. However, the small size of this overshoot (in comparison with the nearly 8 mV surround response elicited with diffuse illumination) suggests that the surround made only a minor contribution to the wave form.

Amacrine and ganglion cells

Amacrine and ganglion cell responses were identified by their characteristic wave forms to flashes of diffuse illumination (Werblin & Dowling, 1969). Amacrine cells responded with transient depolarizations at the onset and cessation of illumination, with one or two small spikes often superimposed on the leading edge of the potentials. Ganglion cells for the most part also responded transiently, but could be either depolarizing or

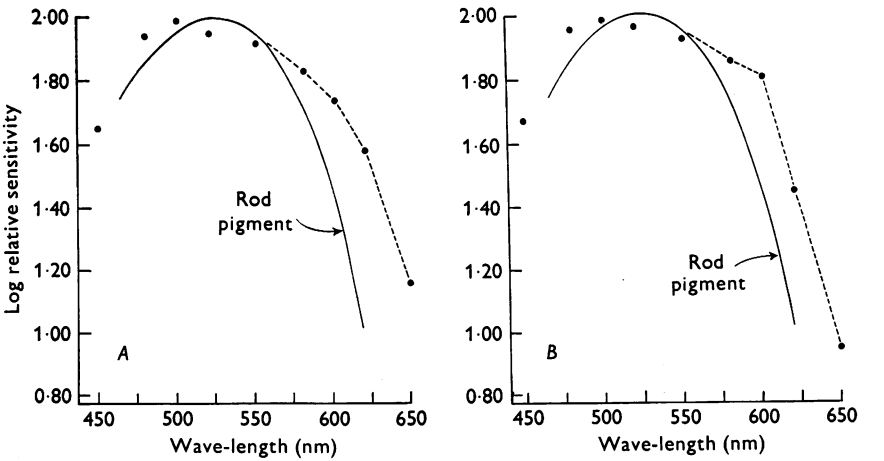

Fig. 19. Spectral sensitivities of dark-adapted amacrine cell (*A*) and hyperpolarizing on–off ganglion cell (*B*) for 190 msec flashes of diffuse light. Both have been fitted at short wave-lengths with the rod pigment log relative absorbance (continuous curves).

hyperpolarizing. The responses of depolarizing cells closely resembled those of amacrines, but the action potentials were somewhat larger and much more numerous, especially at bright light intensities. The responses of hyperpolarizing cells also resembled amacrines but were opposite in sign: they gave negative-going responses at both the onset and cessation of the flash. These cells usually fired spontaneously in the dark, in which case their light responses were characterized by a transient decrease of firing at on and off. However, some cells were quiet in the dark and gave brief bursts of action potentials following each hyperpolarization at its return to the base line.

Spectral sensitivity curves for dark-adapted amacrine and ganglion cells were similar in shape to those of the rod–cone bipolar cells. Representative curves are given in Fig. 19. The amacrine cell (*A*) and the ganglion cell (*B*) were both dominated by rod input in the dark-adapted retina, and their

sensitivities have been fitted with the rod pigment relative absorbance curve. However, they were both more sensitive than the rods to long-wavelength illumination. As for the rod–cone bipolars, this second component is probably coming from the cones. However attempts to demonstrate a shift in the spectral sensitivity in the presence of background illumination were unsuccessful. The absolute latency of the response, which was used to measure the spectral sensitivities of these cells, became much shorter in the presence of the background. This greatly reduced the extent of variation of the latency, thus making reliable measurements of relative sensitivity much more difficult.

DISCUSSION

The principal conclusion of this paper is that rod and cone signals interact in the mudpuppy retina. These interactions are of two kinds. Some neurones (for example, amacrine cells and most horizontal cells) receive signals from both rods and cones which are of the same sign, so that the responses of these cells are 'L-type' or 'non-colour-opponent'. Other cells, like the C-type horizontal cell and some cone bipolars, receive rod and cone signals of different polarities. The reciprocal inhibition of receptor signals in these cells produces 'chromaticity' (C-type) or 'colour-opponent' responses, which are similar to those generated by the signals of cones in vertebrates known to discriminate wave-lengths (Daw, 1973). Both colour-opponent and non-colour-opponent responses are examined in the following.

Rod and cone interactions in horizontal cells

The great majority of horizontal cells recorded from the mudpuppy retina are L-type cells whose spectral properties are similar to those of L-type cells in the cat (Steinberg, 1969*a*, *b*; Niemeyer & Gouras, 1973): cells in both species receive input from cones and from a second process having greater sensitivity to short wave-lengths. This second input apparently comes from the rods, since it is absolutely more sensitive than the cone input (Fain & Dowling, 1973). Furthermore, the responses of L-type cells in both mudpuppy and cat show systematic changes in wave form across the spectrum, which can be attributed to differences in the wave forms of rod and cone receptor responses.

Examination of Golgi-impregnated horizontal cells in cat reveals two classes of cells (Fisher & Boycott, 1974), one of which, type B, is postsynaptic to rods at its axon terminals and to cones at its dendritic endings. Cells morphologically similar to type B horizontals have also been found in rabbit (Fisher & Boycott, 1974) and monkey (Boycott & Kolb, 1973*b*;

Ogden, 1974), and there is evidence in monkey that they also make contacts with both kinds of photoreceptors (Kolb, 1970). Although such cells have not been identified in mudpuppy, horizontals of somewhat different morphology have been shown to make dendritic contacts with both rods and cones in another urodele, the salamander *Ambystoma tigrinum tigrinum* (Lasansky, 1973).

Mudpuppy L-type cells are non-colour-opponent, since the inputs from rods and cones are both hyperpolarizing. The C-type horizontal cells, on the other hand, receive hyperpolarizing input from rods and depolarizing input from cones. The responses of these cells resembled those commonly recorded from colour-opponent horizontal cells in fish and turtle retinas (Svaetichin, 1956; Tomita, 1965; Miller, Hashimoto, Saito & Tomita, 1973), but there were two major differences. First, C-type cells recorded in these latter species receive input from two or three spectral classes of cones, but appear not to receive input from rods (Naka & Rushton, 1966; Fuortes & Simon, 1974). Second, a purely depolarizing response can be elicited in the dark-adapted retina from C-type cells in fish and turtle by a stimulus of the appropriate wave-length. Purely depolarizing responses were not recorded from the mud-puppy C-type cell even to long-wave-length flashes in the presence of short-wave-length background illumination (Fig. 10). This suggests that the responses of C-type cells in these species may not be generated by the same mechanism, but more information will be necessary to explore this question in detail.

Rod and cone interactions in bipolar cells

Mudpuppy bipolar cells receive direct synaptic input from photoreceptors at receptor terminals and indirect input through synapses from horizontal cells (Dowling & Werblin, 1969). The dendritic trees of bipolars are about the same diameter as their receptive field centres (Werblin & Dowling, 1969; Kaneko, 1973), suggesting that the direct input from the photoreceptors produces the centre response. The much larger receptive field of the antagonistic surround is presumably formed by input from horizontal cells. Horizontal cells may also inhibit photoreceptors (Baylor *et al.* 1971), producing a receptor surround antagonism which in the turtle may also make a contribution to the bipolar cell receptive field (Schwartz, 1974). However, this receptor inhibition appears not to be important in the mudpuppy, since surround antagonism is much stronger in bipolars than receptors when the responses of both are compared under identical stimulus conditions (G. L. Fain, unpublished observations; Werblin & Dowling, 1969). Moreover the inhibition of cone signals by rod signals presumably coming from L-type horizontal cells is much greater in bipolar cells (Fig. 15) than in receptors (Fig. 5). It is doubtful that this difference

can be simply attributed to a decrement in horizontal cell inhibition between the receptor synaptic terminal and the site of intracellular recording, since the large diameter of mudpuppy receptors and the short distance between their terminals and the rest of their cell bodies would permit efficient electrotonic conduction (Brown *et al.* 1963). It might be argued that mudpuppy receptors showed weak surround antagonism in these experiments because they were recorded from retinas which were partially anoxic (Baylor *et al.* 1971). However since bipolar cell recordings were made under similar conditions and occasionally from the same retina, it is likely that the surrounds of mudpuppy bipolar cells are produced in great part by synapses from horizontal cells on to the bipolars directly.

Recordings were made from two spectral classes of bipolars, whose dark-adapted centre responses showed either cone (Figs. 12, 13) or rod and cone sensitivity (Fig. 17). These presumably arise from cells whose dendrites are post-synaptic either to cones or to both kinds of receptors. Although no anatomical evidence for these two classes is available for mudpuppy, they have both been shown to be present in the fish retina (family *Cyprinidae*) (Stell, 1967; Scholes, 1975). It is not known whether these morphological classes represent different physiological types in the fish, since spectral measurements have so far been made only in mesopic conditions where they revealed only cone input (Kaneko, 1973). Bipolar cells making contacts with both rods and cones have also been described in the retina of the larval tiger salamander (Lasansky, 1973). However such cells do not appear to be present in mammals, where bipolar cells make contacts exclusively with one kind of receptor or with the other (Boycott & Dowling, 1969; Kolb, 1970; Boycott & Kolb, 1973*a*).

The dark-adapted surround responses of mudpuppy bipolar cells also fell into two classes: those showing predominantly cone sensitivity (Fig. 12) and those showing predominantly rod sensitivity (Fig. 13). These presumably were generated by L-type horizontal cells, with the 'cone' surrounds coming from cells showing little rod sensitivity (Fig. 9) and the 'rod' surrounds from rod–cone horizontals. The 'rod' surrounds were relatively more sensitive than L-type horizontal cells to short wave-lengths (compare Figs. 13 and 6), perhaps reflecting contributions to the surround from C-type horizontal cells. However, this enhanced sensitivity in the blue may have been entirely the result of light scattered into the centre of the bipolar cell receptive field from the annulus used to evoke the surround response. Since the spectral sensitivities of surrounds were measured in cone bipolar cells, scattered light would have produced a central (cone) response which would have diminished the relative sensitivity of the surround to long wave-lengths.

When bipolar cells are stimulated with diffuse light, the centre and surround respond together. If the two parts of the receptive field have the same relative spectral sensitivities as in Fig. 12, then the responses to diffuse illumination will also have this spectral sensitivity and the cell will be non-colour-opponent. If the centre and surround have different spectral sensitivities as in Fig. 13, the cell will be colour-opponent and its spectral sensitivity for diffuse light will be narrower than for central illumination. Chromatic interactions between rod and cone signals in mudpuppy bipolar cells are similar to those observed between the signals of different spectral classes of cones in goldfish bipolars (Kaneko, 1973).

Rod and cone interactions in the inner plexiform layer

Mudpuppy amacrine cells receive input from the outer plexiform layer through synapses at the terminals of bipolar cell axons (Dowling & Werblin, 1969). All the amacrines whose spectral properties were investigated in these experiments showed predominantly rod sensitivity but were consistently more sensitive than rods to long wave-lengths. This increased sensitivity was probably produced by cone input coming from the rod–cone and cone bipolars. This notion is substantiated by spectral measurements of the mudpuppy proximal negative response (Proenza & Burkhardt, 1973), an extracellular field potential arising predominantly from amacrine cells (Burkhardt, 1970). The dark-adapted spectral curve of the proximal negative response in the mudpuppy shows predominantly rod sensitivity but, like the amacrine cells, is somewhat more sensitive than the rods to long wave-lengths. In the light-adapted retina, the sensitivity of the proximal negative response closely follows the cone relative absorbance curve. Hence the increased sensitivity of the proximal negative response and of the amacrine cells to long wave-lengths in the dark is also most likely produced by cone signals.

Ganglion cells receive synaptic input from bipolar cells and from amacrine cells. The mudpuppy retina contains at least two kinds of ganglion cells: a 'phasic' or 'on–off' type whose responses are either depolarizing or hyperpolarizing, and a 'tonic' type whose receptive field has concentric and mutually antagonistic centre and surround regions, the centre responding either with a relatively sustained burst of impulses ('on' unit) or a cessation of spontaneous firing ('off' unit) (Werblin & Dowling, 1969; Werblin, 1970). Spectral measurements were made only from on–off cells, which showed predominantly rod sensitivity but appeared also to be driven by cones. The similarity of the spectral sensitivities in Fig. 19 is consistent with the notion that mudpuppy on–off ganglion cells receive much of their input from amacrine cells (Werblin & Dowling, 1969).

The responses of mudpuppy amacrine and on–off ganglion cells, like those of transient amacrines (Kaneko, 1973) and on-off ganglions (Beauchamp, 1974) in the goldfish, are non-colour-opponent. The goldfish also contains other amacrine cells which have simple, undifferentiated receptive fields but which give sustained, colour-opponent responses (Kaneko, 1973). Cells of this kind were not recorded from the mudpuppy.

If inhibitory interactions of rod and cone signals are used by the mudpuppy for wave-length discrimination, they must be transmitted to ganglion cells and then to higher visual centres in the brain. Colour-opponent ganglion cells in the goldfish (Wagner, MacNichol & Wolbarsht, 1960; Daw, 1968), ground squirrel (Michael, 1968) and monkey retinas (Gouras, 1968) are typically tonic units with antagonistic centre–surround receptive fields. Occasional recordings were made from tonic ganglion cells in mudpuppy, but it could not be determined whether they were colour coded since it was not possible to record from them long enough to characterize their spectral properties. Hence it cannot yet be said whether the chromatic interactions observed in C-type horizontal cells and bipolar cells in the mudpuppy are actually utilized for making colour discriminations.

Rod and cone interactions and visual perception

The strong interactions between rod and cone signals which have been observed in mudpuppy retinal interneurones may seem surprising, since it is usually assumed that rods and cones do not interact to influence visual behaviour. Although the signals of the two kinds of photoreceptors are functionally independent under a variety of stimulus conditions in man (Stiles, 1944; Alpern, 1965; Westheimer, 1970) and other vertebrates (Gouras & Link, 1966; Daw & Pearlman, 1969), rod and cone interactions can be detected psychophysically (Hollins, 1971; Frumkes *et al.* 1972; Makous & Boothe, 1974). Moreover, rods are able to participate in human colour perception, since the stimulation of only the rods and long-wavelength cones can produce a variety of colour sensations in dark-adapted observers (Willmer, 1949; McCann & Benton, 1969).

Rod and cone signals could interact in the human retina along pathways similar to those described above for the mudpuppy. Primates have horizontal cells which make contacts with both rods and cones (Kolb, 1970; Boycott & Kolb, 1973*b*; Ogden, 1974), and these presumably inhibit either photoreceptors or bipolar cells or both to produce antagonistic surround responses. Since primate bipolar cells are connected exclusively to rods or to cones (Boycott & Dowling, 1969; Kolb, 1970), the inhibition of rod–cone horizontal cells would produce colour-opponent responses in bipolars. Some primate horizontal cells could themselves be colour-

opponent like mudpuppy C-type cells. Whether pathways of this kind do in fact mediate interactions between rod and cone signals in the human retina remains to be demonstrated.

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